Detection and Forensic Analysis of Wildlife and Zoonotic Disease

Brucellosis is endemic to bison and elk populations in the Greater Yellowstone Area.

INL is developing assays and techniques that will facilitate detection and molecular fingerprinting of high consequence pathogens. Current work focuses on the detection and forensic analysis of *Brucella* species, which are pathogens responsible for disease in a broad spectrum of animal and human hosts. Concerns over the possible use of *Brucella* species as agents of biological warfare targeting humans or domestic animals, specifically cattle, exist. In addition, this research will contribute to understanding the potential for natural transmission of brucellosis from bison and elk populations (in which the disease is endemic), to domesticated cattle in the Greater Yellowstone Area. Reagents developed at INL will thus have value not only to national biodefense, but also to national and regional animal husbandry, and wildlife management issues that affect U.S. agricultural security.

The project will generate a unique set of validated (against real-world diagnostic and environmental samples) DNA signatures, for the closely related Category B select agents, *Brucella abortus*, *B. melitensis* and *B. suis*. A variety of techniques will be employed. INL molecular microbiologists have developed a real-time (fluorescence-based) polymerase chain reaction (PCR) test that allows detection of active *Brucella abortus* infection in bison, other wildlife and cattle in approximately 30 minutes. This is an improvement over conventional (gel-based) PCR which typically requires about three hours for assay results. INL has a field-portable real-time PCR instrument allowing the assay to be run in the field at trap sites. Additional real-time PCR assays are being developed and validated to target other species, incorporate internal controls, and allow multiplexing (detection of more than one target in a single reaction). While real-time PCR is rapid and sensitive, it may not afford a suitable platform to perform strain typing, particularly within the *Brucella*, which are genetically homogeneous across species and strains. Accordingly, scientists are developing appropriate methods and instrumentation to perform rapid, high-throughput microbial forensic analysis of samples for identification at the strain or isolate level. By combining sets of highly discriminatory primers to amplify and label repetitive or

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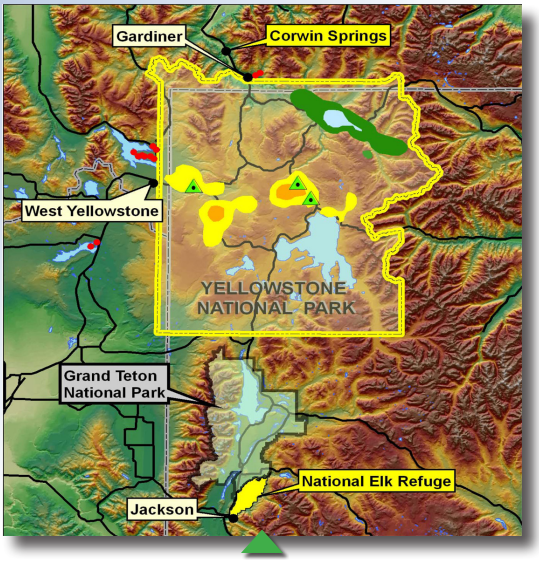

Distribution of the northern (green) and central (yellow) bison herds within Yellowstone National Park. Red indicates seasonal migration outside of the park boundaries. Green triangles indicate sites where samples have been taken for real-time PCR and cultivation analyses.

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Field-portable real-time PCR instrument called the Ruggedized Advanced Pathogen Identification Device (RAPID).

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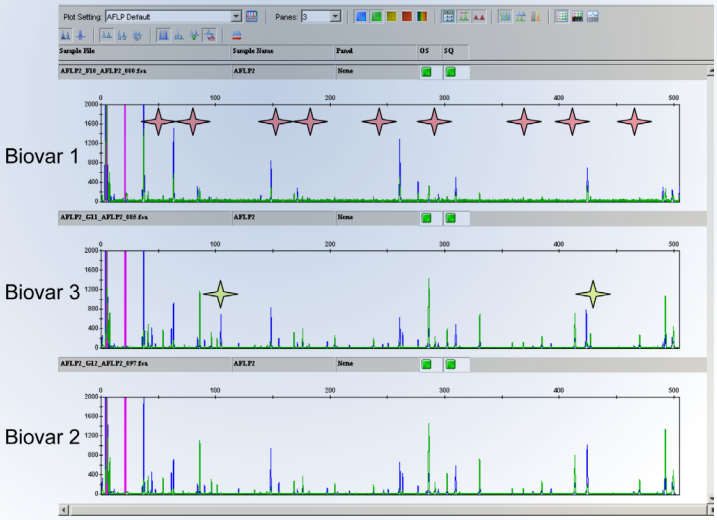
unique sequences from the target organism's DNA with high-throughput, high-resolution capillary electrophoresis, they will develop a means of handling large numbers of samples. Strain typing of pathogenic strains by molecular methods is important to epidemiological and forensic studies. Methods include pulsed field gel electrophoresis of large chromosomal restriction fragments, insertion element number and restriction fragment length polymorphisms (RFLP), rRNA RFLP patterns (ribotyping), amplified fragment length polymorphisms (AFLP), and analysis of variable-number tandem repeats (VNTR).



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Selected Publications/Presentations

Foster, J.T., S.M. Beckstrom-Sternberg, T. Pearson, J.S. Beckstrom-Sternberg, P.S.G. Chain, J. Hnath, F.F. Roberto, T. Brettin, and P. Keim, 2009. Whole genome-based phylogeny and divergence of the genus *Brucella*. *J. Bacteriol*. 191, 2864-2870.

Newby, D.T., T.L. Hadfield, and F.F. Roberto. 2003 Real-time PCR detection of *Brucella abortus*: a comparative study of SYBR Green I, 5'-exonuclease, and hybridization probe assays. Appl. Environ. Microbiol. 69:4753-4759.

Roberto, F.F., H.G. Silverman, and D.T. Newby. Comparison of genotyping methods for Brucella. American Society for Microbiology General Meeting, Orlando, FL, May 22, 2006, Poster Z-020.

Roberto, F.F. and D.T. Newby. Deliberate release or natural outbreak? Challenges facing rapid detection methods for zoonotic disease like brucellosis. R&D Partnerships in Homeland Security, Boston, MA, April 27-28, 2005.

Roberto, F.F. and D.T. Newby. Detection of *Brucella abortus* in soils by real-time PCR. Annual Meeting, American Society for Microbiology, New Orleans, LA, May 23-27, 2004, Poster Q-229.

Newby, D.T. and F.F. Roberto. Real-time PCR assay for field diagnosis of *Brucella abortus* in wildlife populations in Yellowstone National Park. Brucellosis 2003 International Research Conference, University of Navarra, Pamplona, Spain, September 15-17, 2003.